Palynology of different populations of *Juniperus polycarpos* complex in Iran

Fatemeh Hojjati[1]

[1]Department of Plant Biology, Faculty of Biological Sciences, Tarbiat Modares University, Tehran 14115-154, Iran. Fatemeh.hojjati@modares.ac.ir

ABSTRACT

Analyses of *Juniperus* in Iran, which have been treated as *Juniperus excelsa* in Iran, using morphology, isoenzyme, terpenoids and DNA sequences, revealed that two cryptic, genetically distinct but morphologically almost identical species are distributed in the country. These two species are *J. polycarpos* with two varieties distributed in N and *J. seravschanica* distributed in SE Iran. The most important character in diagnosing these species is the thickness of the ultimate branchlets. As micro morphological characters, all pollen grains were spherical, monoporate with echinnate orbicules as sculpture elements. Based on the pollen morphology we can observe the shortest pollen grains orbicules echinae and, relatively, the biggest pollen grains and pores in populations from S Iran as *J. seravschanica*. Published on-line www.phytologia.org *Phytologia 101(1): 67-73 (March 21, 2019).* ISSN 030319430.

KEY WORDS: cryptic species, pollen grains, micro morphological character, *J. polycarpos* complex, *J. seravschanica,* Cupressaceae, SEM, Iran.

The genus *Juniperus* (Cupressaceae) is composed of approximately 75 species in 3 monophyletic sections (Mao et al. 2010, Adams and Schwarzbach 2013): *Caryocedrus* with one species, *Juniperus*, with 14 species and *sabina* with 60 species. Among these species, *J. excelsa* M. Bieb of sect. *Sabina* and relative taxa (referred here as *J. excelsa* complex) form a taxonomically difficult group, as the borders between taxa cannot be sharply defined by morphology. According to the latest study, this complex consists of three morphologically very similar species as: *J. excelsa*., *J. polycarpos* K. Koch. (var. *polycarpos* Linnaea and var. *turcomanica* R.P. Adams) and *J. seravschanica* Kom. (Hojjati and Adams unpublished) In Iran *J. polycarpos* is distributed in N with two varieties: *polycarpos* and *turcomanica*, *J. seravschanica* is distributed in SE and hybrid samples are distributed in SW of the country and there is no *J. excelsa* (Hojjati et al. 2018).

However there are different treatments on this group depends on the taxonomic value that each botanist arbitrarily attributed to these taxa (Marschal von Bieberstein, 1800, 1808; Koch, 1849; Boissier, 1884; Fedtschenko et al., 1932; Komarov, 1932; Riedel, 1968; Farjon, 1992; Assadi, 1998; Adams, 1999, 2014). Some populations of the Iran *J. excelsa* complex have been studied.

Based on isoenzyme data, Hojjati *et al.* (2009) recognized 3 major clusters as *J. polycarpos* var. *polycarpos*, *J. polycarpos* var. *turcomanica* and *J. seravschanica* of this complex in Iran. Adams and Shanjani (2011), using DNA sequence data, showed the juniper from the Elburz Mtns. to be typical *J. polycarpos* not *J. excelsa*. Subsequently, Adams and Hojjati (2012) and Adams *et al.* (2014) employing four DNA regions (nrDNA, *pet*N-*psb*M, *trn*D-*trn*T and *trn*S-*trn*G, 3,705 nucleotide site) showed that the samples from NW Iran are *J. polycarpos* and samples from NE Iran are clearly *J. polycarpos* var. *turcomanica*, as are the samples from Fasa in SW Iran. The samples from nearby southcentral Iran (Khabr protected area) are part of a clade with *J. seravschanica* (Komarov) Kitamura.

Several studies on the pollen morphology of members of Cupressceae have been published. An initial overview of the pollen morphology of this family was provided by Erdtman (1965). Hyde and Adams (1958), Kapp (1969), Bassett et al. (1978) and Ciampolini and Cresti (1981) reported that pollen grains of *Juniperus* are inapeturate, but Nilsson et al. (1977), Moor and Webb (1978), Lewis et al. (1983) and Bortenschlager (1990) described monoporate pollen grains in *Juniperus*. With respect to pollen sculpture, it has been generally stated that the surface of pollen grains is irregularly scabrate and is covered by irregularly arranged orbicules (Huiho and Sziklai, 1973; Pocknall, 1981; Bortenschlager, 1990; Yu, 1997). These studies indicate high similarity and uniformity of pollen structure among different taxa of Cupressaceae.

The present study compared *J. polycarpos* and *J. seravschanica* micromorphologically to determine if they have any differences in their pollen exine.

MATERIALS AND METHODS

Twelve populations of *J. polycarpos* complex in Iran were sampled. The voucher specimens were deposited in the Central Herbarium of Tehran University, TUH. Population name, location, herbarium number, longitude, latitude and altitude for each population were listed in Table 1.

For the micro morphological study, pollen grains of 12 populations of *J. polycarpos* complex in Iran were studied by light microscope (LM) and scanning electron microscope (SEM). The pollen samples were obtained from both fresh and dried herbarium specimens and then prepared following rhodaniden method described by Tatzreiter (1985). In comparison with the Erdtman's acetolysis method that splits pollen grains into two halves, the gentler rhodanid method prevents the occurrence of artifacts in pollen grains so that they remain almost without exception in their original spherical form. In rhodaniden method, briefly, we used two solutions: solution A that is composed of HCl, $MgCl_2$, $MgSO_4$, $AlCl_3$ and $AlPO_4$ and solution B that contains KSCN or NaSCN. The prepared materials provided specimens for investigations on LM as well as SEM. Slides for LM were studied and photographed. The diameter of spherical pollen grains and exine thickness were measured and with aid of a ×100 eyepieces. Measurement of pollen grains was based on 30 grains per sample. For SEM study the pollen grains were put on stubs and sputter coated with approximately 25 nm of gold-palladium (Au-Pd) alloy and then scanned in a Vega SEM model VG2080573IR at 15 kV.

RESULTS AND DISCUSSION

Nine quantitative and qualitative pollen morphological characters studied in 12 populations are listed in Table 2. All pollen grains surveyed were spherical and monoporate so that a circular pore situated at the distal pole. The diameter of this pore was consistently less than 2 μm. The exine elements were spherical shaped orbicules that were isolated or in larger or smaller groups. It appeared that the arrangement of these elements was generally looser at the proximal pole. Their surfaces were themselves occupied with small echinae. Figures 1 (1-12) show some photographs of the studied pollen grains. The mentioned features utilized for pollen morphology in this study were as below. Pollen diameter ranged from 26.2 μm in Lushan1 population to 31 μm in Fasa and showed the highest amounts in most of the southern populations. Exine thickness was mostly stable at 1 μm but was 1.2 μm in Qushchi population. Pollen grain pore diameter varied from 0.5 μm in Shahmirzad to 1.8 μm in Genu population. In this case all of the southern populations showed higher values than most of the other populations. Sculpture regulation as a qualitative character was mostly stable with low variation between populations, the same pattern was also found for sculpture density. Sculpture amount had 3 values for variations. Orbicules diameter ranged from 0.7 μm to 1 μm in Fasa population. Annulus existed around the pollen grains pore only in a few populations (Fig. 8). Orbicules echinae length differs among populations studied here so that it was middle in northwestern populations, high in north and northeastern ones and low in southern

populations (Figs. 3, 6, 9 and 12). Based on the pollen morphology we can observe the shortest pollen grains orbicules echinae, relatively, the biggest pollen grains and pores in southern populations.

Table 1. Location of populations of *Juniperus polycarpos* complex studied in Iran.

Population name	**Species**	**Location**	**Herbarium number**	**Lat., Long.**	**Elev (m)**
Lushan1	*J. polycarpos* var. *polycarpos*	N Iran, Prov. Gilan, Lushan to Jirandeh, 15 km to Jirandeh.	33606	36 ° 40′ N, 49° 38′ E	1100-1120
Lushan2	*J. polycarpos* var. *polycarpos*	N Iran, Prov. Gilan, 5 km after Jirandeh towards Yeilaq and Amarlu.	33607	86° 40′ N, 49° 42′ E	1670-1690
Hashtjin	*J. polycarpos* var. *polycarpos*	N Iran, Prov. Ardebil, 16 km towards Hashtjin after Khalkhal.	33608	37° 26′ N, 48° 24′ E	1590-1610
Qushchi	*J. p.* var. *polycarpos*	N Iran, Prov. Azerbaijan, 28 km to Salmace after Orumiee.	33609	38° 0.1′ N, 44° 57′ E	1730-1800
Shahmirzad	*J. polycarpos* var. *turcomanica*	N Iran, Prov. Khorasan, 15 km after Shahmirzad towards Fooladmahaleh.	33610	35° 50′ N, 53° 26′ E	2422
Bajgiran	*J. polycarpos* var. *turcomanica*	N Iran, Prov. Khorasan. 35 km to Bajgiran.	33611	37° 25′ N, 58° 32′ E	1868
Golestan	*J. polycarpos* var. *turcomanica*	N Iran, Prov. Gorgan, Golestan National Park, Sharleq, 9 km towards Azadshahr.	33612	37° 19′ N, 56° 2′ E	1055
Balade	*J. polycarpos* var. *turcomanica*	N Iran, Prov. Mazandaran, 10 km after Baladeh towards Kujur.	33613	36° 14′ N, 51° 50′ E	2924
Fasa	*J. polycarpos* X *J. seravschanica*	S Iran, Prov. Fars, 30 km after Fasa towards Neiriz.	33614	29° 9′N, 53° 40′ E	1607
Kuhbanan	*J. seravschanica*	S Iran, Prov. Kerman, Kuh-e Bajgen, 55 km towards Kuhbanan, Dolatabad village.	33615	31° 28′ N, 55° 52′ E	2091
Khabr	*J. seravschanica*	of Iran, Prov. Kerman, Kuh-e Khabr.	33616	28° 51′ N, 56° 22′ E	2418
Genu	*J. seravschanica*	S Iran, Prov. Kerman, Kuh-e Genu	33618	27° 24′ N, 56° 11′ E	1673

Table 2. Nine quantitative and qualitative micro morphological characters studied in 12 populations of Iran *Juniperus polycarpos* complex. A= irregular B= relatively regular C= mostly isolated D= mostly clustered E= low F= middle G= high H= presence I= absence J= short K= medium L= long

Populatiion, taxon	**Characters**								
	Pollen grain diam. (µm)	Exine thickness (µm)	Pore diam. (µm)	Orbicules regulation	Orbi-cules density	Orbi-cules amount	Orbi-cules diam. (µm)	Existence of annulus around the pore	Orbi-cules echinae length
Lushan1 *J. p.* var. *polycarpos*	24.0 26.2 29.0	1.0	0.8	B	C	F	0.8	I	K
Lushan2 *J. p.* var. *polycarpos*	25.0 27.0 30.0	1.0	1.2	A	D	F	0.8	I	K
Hashtjin *J. p.* var. *polycarpos*	26.0 27.6 29.0	1.0	1.0	A	D	G	0.7	I	K
Qushchi *J. p.* var. *polycarpos*	27.0 29.0 31.0	1.0 1.2 2.0	1.1	A	D	G	0.9	I	K
Shahmirzad *J. p.* var. *turcomanica*	26.0 28.0 30.0	1.0 1.1 2.0	0.5	A	D	E	0.7	I	L
Bajgiran *J. p.* var. *turcomanica*	26.0 29.3 33.0	1.0	1.4	A	D	G	0.7	I	L
Golestan *J. p.* var. *turcomanica*	27.0 28.6 32.0	1.0	1.2	B	C	G	0.9	H	L
Balade *J. p.* var. *turcomanica*	26.0 26.8 29.0	1.0	1.7	A	D	E	0.7	H	L
Fasa *J. polycarpos X J. seravschanica*	27.0 31.0 34.0	1.0	1.4	A	D	E	1.0	H	J
Kuhbanan *J. seravschanica*	27.0 30.0 32.0	1.0	1.5	A	D	G	0.8	I	J
Khabr *J. seravschanica*	26.0 29.0 32.0	1.0	1.6	A	D	G	0.8	I	J
Genu *J. seravschanica*	26.0 29.4 33.0	1.0	1.8	A	D	E	0.8	H	J

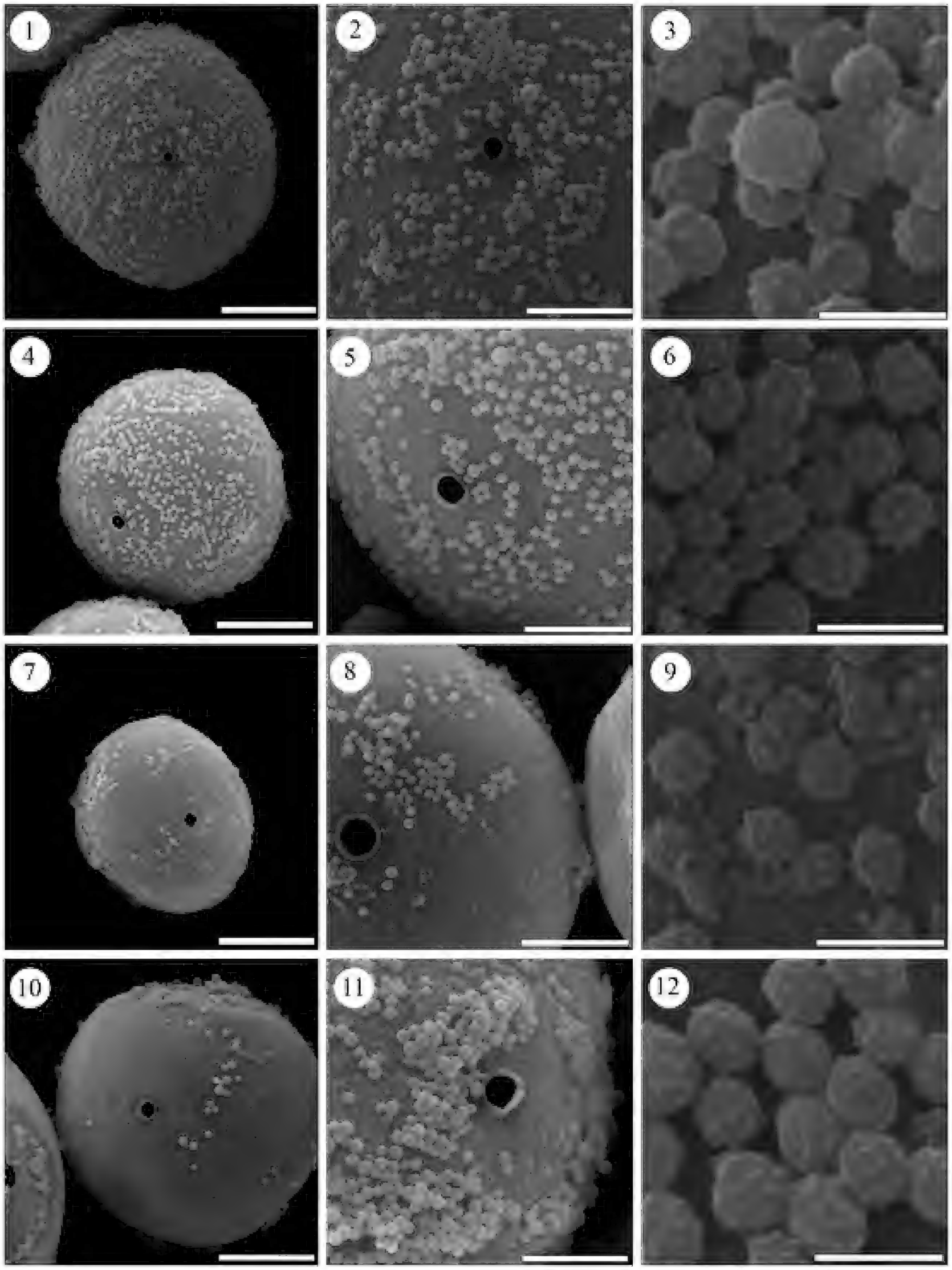

Fig.1. 1-12, SEM micrographs of pollen grains in some of *Juniperus polycarpos* complex Iran populations. Figs. 1 and 2, Pollen grain from Qushchi population. Fig. 3, Pollen orbicules echinae from Hashtjin. Figs. 4 and 5, Pollen grain from Golestan. Fig. 6, Pollen orbicules echinae from Bajgiran. Figs. 7 and 8, Pollen grain from Balade. Fig. 9, Pollen orbicules echinae from Balade. Fig. 10, Pollen grain from Fasa. Fig. 11, Pollen grain from Khabr. Fig. 12, Pollen orbicules echinae from Genu. Bar scale 1, 4, 7, 10=10 µm, 2, 5, 8, 11=5 µm and 3, 6, 9, 12=1 µm.

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